The control of reproductive cycles in higher primates is largely dependent on negative and positive feedback mechanisms by both steroidal and non-steroidal substances of the ovaries which regulate the function of hypothalamo-pituitary system. To gain insights into the role of INH A, the non steroidal ovarian hormone in the feedback control of pituitary FSH secretion, studies were conducted to examine the interrelationships of hormones throughout the menstrual cycle of the bonnet macaque. The findings of chapter II provide a detailed description of endocrine hormone profile during the menstrual cycle of the bonnet macaques with special attention to the feedback role of INH A on pituitary FSH secretion. To characterize the endocrine profile of different hormones, both ovarian (E_2, P_4, INH A) and pituitary (FSH, LH) hormones were measured daily for more than 40 days. To further examine the site of secretion of INH A and its relationship with pituitary FSH dynamics, surgical lutectomy and pharmacological induction of luteolysis employing the third generation GnRH R antagonist, Cetrorelix (CET) studies were carried out in the subsequent experiments. The results obtained from these studies suggest that INH A and P_4 secreted from the CL during luteal phase regulate pituitary FSH secretion. The selective rise in FSH observed during the late menstrual cycle and during menstruation (referred to as luteo-follicular transition), as has been reported previously in higher primates, considered necessary for initiation of follicular growth and recruitment of follicles for ensuing menstrual cycle was characterized in the monkey. Surgical lutectomy and induction of luteolysis by CET experiments suggested that increased GnRH secretion is essential for this selective rise in FSH following withdrawal of inhibition by P_4 and INH A. In clinical cases of reproductive ageing, the shortened follicular phase in human females has been identified to be the result of occurrence of early onset of FSH rise during the luteal-follicular transition period. The cause(s) of declining fertility with age in women who still have regular menstrual cycles are not clear, but issues of relationship between dysregulation of selective FSH rise in the late luteal phase and associated infertility could be examined using bonnet monkey as a model system.

INH A is secreted in significant quantities by CL in higher primates and the feto placental unit suggesting its importance during fertility and pregnancy. Apart from the

negative feedback regulation of pituitary FSH secretion, the complete repertoire of actions of this hormone during pregnancy is yet to be fully understood. The data presented in this thesis is the first comprehensive report showing the endocrine hormone profile of gonadotropins and ovarian hormones including INH A throughout the menstrual cycle of the bonnet macaque. The characterization of INH A profile in bonnet monkey will be of significant value for studies examining the role of INH A in higher primates. Dimeric inhibin has been suggested to be important for regulation of fertility and reproductive functions. Also, inhibin-α (one of the subunits of the dimeric protein) knock out mice model has provided convincing evidence that it acts as a tumour suppressor. A great deal of new information has been generated in recent years regarding the potential clinical usefulness of monitoring inhibin levels in blood and biological fluids in gynaecological diseases, pathological pregnancies and other disorders. Emerging clinical roles of inhibin have made INH A an important candidate molecule to study its molecular regulation. The results presented in chapter II suggested that LH regulates luteal INH A secretion (induction of luteolysis by CET administration experiment). As a first step towards understanding molecular regulation of inhibin-α expression in the macaque CL, in silico promoter analysis of macaque inhibin-α was performed and it revealed several transcriptional factor binding sites that were conserved across species. In rats FSH up regulates while preovulatory LH surge suppresses inhibin-α mRNA expression in the ovary and this suppression has been suggested to be necessary for occurrence of secondary FSH surge during metestrus. To address differential regulation of inhibin-α by LH and FSH in rat ovary during the periovulatory period, studies employing immature rats were carried out and the results are presented in chapter III. The results suggest that immature rat ovaries respond to exogenous gonadotropins in terms of LH signaling (cAMP production), luteinization (P_4 production) and as well induction of ICER expression required for repression of inhibin-α subunit expression. PDE4 inhibitor (rolipram) treatment enhanced the ovarian cAMP concentrations suggesting that PDE4 play a major role in controlling intraovarian cAMP concentrations in rat ovaries. However increased cAMP concentrations did not appear to up regulate the ICER expression at the time point examined in this study.

In higher primates time course of second FSH surge and continued synthesis and secretion of INH A in the CL are different from non primate species. In the monkey, the second FSH rise occurs during the late luteal phase and experiments have been carried out to examine the regulation of inhibin-α subunit expression by ICER. Expressions of ICER (mRNA/protein) and INH A were examined during different stages of CL and the results indicated no clear inverse relationship between the ICER and inhibin-α mRNAs. With no conclusive role for the ICER in regulating luteal inhibin-α observed in the study, the role of transcriptional activators in the regulation of inhibin-α like GATA4, SF-1, β-catenin were further examined. Since luteal INH A secretion was dependent on pituitary LH as determined earlier in chapter II, expressions of transcriptional activators were examined in CL of different stages and also during induced luteolysis and the results are described in chapter IV. In conclusion, our results indicate cross talk between WNT, cAMP and P38 MAP kinase signaling pathways in the regulation of luteal INH A secretion.

The pituitary gonadotropin, LH, is the primary luteotropin in primate species acting to maintain the structure and function of the CL during the menstrual cycle. However whether the actions of LH are direct or mediated by local factors such as P_4 remain unknown. Moreover, P_4 secretion which is dominant during luteal phase has any role in regulating CL structure and function is not clearly defined. To address these and issues concerning P_4 actions, initially, experiments were performed in the rat model to study the importance of P_4 in the regulation of ovarian functions. An antiprogestin, RU486, was employed as a tool to uncover the PR regulated pathways during ovulation in rats and the findings are presented in the chapter V. The results indicated that blockade of PR action by RU486 during gonadotropin-induced superovulation resulted in inhibition of follicular rupture and ovulation in immature rats. Further to understand the downstream effectors of PR action, and to identify the candidate target genes of PR activation, semi-quantitative RT-PCR and western blot analyses were performed. The results obtained indicated that betacellulin, a member of EGF family and MMP-9 a proteolytic enzyme, were markedly repressed in response to RU486 treatment in rat ovaries. Also, the down stream pathway of EGF signaling leading to activation of ERK was

markedly repressed in RU486 treated ovaries. It was next examined what role the P_4/PR system has in the regulation of CL structure and function. Surprisingly, PR expression is absent in CL of rats, while it is present in higher primates. Experiments were carried out to examine intracrine actions of P_4 in the regulation of CL structure and function in monkeys. The recently reported model system of induced luteolysis yet capable of responsive to trophic support from the laboratory provided an ideal opportunity to examine direct effects of P_4 on structure and function of CL in the monkey. A series of pilot experiments were carried out in monkeys experiencing summer amenorrhea, to determine dose and mode of administration of exogeneous P_4 to simulate mid luteal phase circulating P_4 concentrations in monkeys subjected to induced luteolysis. Based on the results of pilot experiments, implantation of Alzet pumps containing 97.5mg of P_4 was selected for maintaining mid luteal phase P_4 concentrations. The microarray data of induced luteolysis previously deposited by the laboratory in NGBI's gene expression omnibus were mined for identification and validation of differentially expressed genes of PR and its target genes following LH depletion and LH replacement experiments. Expressions of PR, PR cofactors and expressions of PR downstream target genes through out the luteal phase and in CL from day1 of menses were also examined. Analysis of expressions of genes revealed that of the 45 genes identified to be regulated by LH treatment, 4 genes were found to be responsive to P_4, and 14 were identified to be responsive to both P_4 and LH. Morphology of CL tissue sections revealed that P_4 treatment appeared to have reversed the induced-luteolysis changes. In another experiment, implantation of P_4 during late luteal phase (i.e., the period of declining P_4 concentrations) for 24h caused changes in expressions of genes associated with tissue remodeling and morphology of luteal cells. Taken together, the results suggest that induced luteolysis plus P_4 replacement model is suitable for assessing the effects of P_4 on CL function. The results also suggest that CL could serve as target tissue for examining the genomic and non genomic actions of P_4.

In summary, studies carried out in the present thesis provides a comprehensive endocrine hormone profile throughout the menstrual cycle of the bonnet monkey with special emphasis on time course of INH A and FSH secretion which is very useful for

future investigations. Studies have been carried out in rats and monkeys with different experimental model systems to address molecular mechanisms underlying inhibin-α regulation in the ovary in general and CL in particular. Experimental findings in monkeys could help elucidate the underlying molecular nature of CL functionality and extrapolate to understand luteal insufficiency and infertility producing conditions in humans. Also different model systems have been validated to examine the actions of P_4/PR system in rats and monkeys and more importantly to address the direct effects of P_4 upon monkey CL structure and function were established. Future investigations based on findings of these studies should help clarify relative roles for LH and P_4 during maintenance of CL function and luteolysis.